

Adiponectin-stimulated CXCL8 release in primary human hepatocytes is regulated by ERK1/ERK2, p38 MAPK, NF- κ B, and STAT3 signaling pathways

Josef Wanninger,¹ Markus Neumeier,¹ Johanna Weigert,¹ Sabrina Bauer,¹ Thomas S. Weiss,² Andreas Schäffler,¹ Corinna Krempl,¹ Cornelia Bleyl,¹ Charalampos Aslanidis,³ Jürgen Schölmerich,¹ and Christa Buechler¹

Departments of ¹Internal Medicine I and ²Surgery and Center for Liver Cell Research, ³Institute of Clinical Chemistry and Laboratory Medicine, University Hospital of Regensburg, Regensburg, Germany

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Wanninger J, Neumeier M, Weigert J, Bauer S, Weiss TS, Schäffler A, Krempl C, Bleyl C, Aslanidis C, Schölmerich J, Buechler C. Adiponectin-stimulated CXCL8 release in primary human hepatocytes is regulated by ERK1/ERK2, p38 MAPK, NF- κ B, and STAT3 signaling pathways. *Am J Physiol Gastrointest Liver Physiol* 297: G611–G618, 2009. First published July 16, 2009; doi:10.1152/ajpgi.90644.2008.—Adiponectin is believed to exert hepatoprotective effects and induces CXCL8, a chemokine that functions as a survival factor, in vascular cells. In the current study, it is demonstrated that adiponectin also induces CXCL8 expression in primary human hepatocytes but not in hepatocellular carcinoma cell lines. Knock down of the adiponectin receptor (AdipoR) 1 or AdipoR2 by small-interfering RNA indicates that AdipoR1 is involved in adiponectin-stimulated CXCL8 release. Adiponectin activates nuclear factor (NF)- κ B in primary hepatocytes and pharmacological inhibition of NF- κ B, the p38 mitogen-activated protein kinase, and extracellular signal-regulated kinase (ERK) 1/ERK2 reduces adiponectin-mediated CXCL8 secretion. Furthermore, adiponectin also activates STAT3 involved in interleukin (IL)-6 and leptin-mediated CXCL8 induction in primary hepatocytes. Inhibition of JAK2 by AG-490 does not abolish adiponectin-stimulated CXCL8, indicating that this kinase is not involved. Pretreatment of primary cells with “STAT3 Inhibitor VI,” however, elevates hepatocytic CXCL8 secretion, demonstrating that STAT3 is a negative regulator of CXCL8 in these cells. In accordance with this assumption, IL-6, a well-characterized activator of STAT3, reduces hepatocytic CXCL8. Therefore, adiponectin-stimulated induction of CXCL8 seems to be tightly controlled in primary human hepatocytes, whereas neither NF- κ B, STAT3, nor CXCL8 are influenced in hepatocytic cell lines. CXCL8 is a survival factor, and its upregulation by adiponectin may contribute to the hepatoprotective effects of this adipokine.

adiponectin; hepatocyte; nuclear factor- κ B; adiponectin receptor 1; STAT3; extracellular signal-regulated kinase; mitogen-activated protein kinase

ADIPONECTIN IS AN adipocyte-derived protein with antidiabetic and hepatoprotective properties, but systemic levels are reduced in obesity, hepatic steatosis, and liver fibrosis (1, 32, 45, 48). Adiponectin receptors 1 (AdipoR1) and 2 (AdipoR2) are expressed in hepatocytes (23, 24, 44) and mediate the hepatoprotective effects of adiponectin, but contradictory data have been published regarding the abundance of adiponectin receptors in injured liver (2, 23, 39). Binding of adiponectin to AdipoR2 activates the peroxisome proliferator activated recep-

tor- α thereby stimulating β -oxidation, whereas AdipoR1 is involved in the activation of the AMP-activated protein kinase, p38 mitogen-activated protein kinase (MAPK), and nuclear factor (NF)- κ B (34, 44). Both receptors activate extracellular signal-regulated kinase (ERK) 1/2 in Hek293 cells through a Src/Ras pathway and stimulate proliferation (16).

In animal models, it has been shown that adiponectin is protective in endotoxin- and concanavalin A-induced hepatotoxicity (19, 43). Adiponectin ameliorates inflammation by lowering the release of proinflammatory cytokines; it inhibits the activation of hepatic stellate cells and antagonizes hepatocytic cell death (19, 32, 43).

CXC chemokines act as survival factors, and adiponectin induces CXCL8, a chemokine that exerts mitogenic and antiapoptotic functions, in monocytes and endothelial cells (29, 40). CXCL8 administration protects mice against concanavalin A-induced hepatitis by inhibiting apoptosis and growth arrest of hepatocytes (26). Similar protective mechanisms have been observed in mice with hepatic overexpression of the CXCL8 ortholog KC where liver injury was induced by galactosamine and endotoxin (8). KC is upregulated by the hepatoprotective cytokine interleukin (IL)-6 via the activation of the IL6-gp130-STAT3 pathway, and recombinant KC reduces serum aminotransferase levels in mice with concanavalin A-mediated liver damage (14).

Besides these antiapoptotic and hepatoprotective effects, CXCL8 is a chemoattractant and activator for neutrophils, basophils, and T cells that may aggravate liver fibrosis (28). Despite 100-fold elevated KC serum levels in the IL-6-treated mice, an influx of polymorphonuclear cells in the liver was not observed (14).

Systemic CXCL8 is elevated in obesity (33) and patients with nonalcoholic fatty liver disease (NAFLD) and is suggested to contribute to the pathogenesis of these disorders (10). NAFLD is often referred to as the hepatic manifestation of the metabolic syndrome, and excess hepatic lipid storage represents a first hit that predisposes the liver to subsequent damage (37). Incubation of hepatocytes with palmitic acid may be used as an in vitro model for hepatic steatosis and, despite an induction of CXCL8 in the lipid-loaded cells, an enhanced apoptosis was observed (12).

Taken together, these data indicate that the function of CXCL8 has not been fully characterized, and its tightly controlled activity may be essential to ensure the protective effects of this chemokine. We have recently shown that adiponectin induces CXCL8 in primary human monocytes by a pathway

Address for reprint requests and other correspondence: C. Buechler, Department of Internal Medicine I, University Hospital of Regensburg, Regensburg 93042, Germany (e-mail: christa.buechler@klinik.uni-regensburg.de).

involving the p38 MAPK, and similar results were published for endothelial cells (29, 40). In the current study, our goal was to investigate whether adiponectin enhances CXCL8 release in hepatocytes and to identify the signaling pathways involved.

MATERIALS AND METHODS

Culture media and reagents. Dulbecco's modified Eagle medium (DMEM) was from Lonza (Wuppertal, Germany), the RNeasy Mini Kit was from Qiagen (Hilden, Germany), and oligonucleotides were synthesized by Metabion (Planegg-Martinsried, Germany). Light-Cycler FastStart DNA Master SYBR Green I was purchased from Roche (Mannheim, Germany). Recombinant human full-length adiponectin expressed in a mammalian cell line, recombinant IL-6, and the DuoSet enzyme-linked immunosorbent assay (ELISA) Development System for human adiponectin were from R&D Systems (Wiesbaden-Nordenstadt, Germany). CXCL8 ELISA was from BD Biosciences (Heidelberg, Germany). Anti- β -actin antibody was ordered from Sigma (Deisenhofen, Germany). NF- κ B p65 antibody was from Acris (Hiddenhausen, Germany), and STAT3, phosphor (P)-STAT3 (Tyr⁷⁰⁵), p38, phospho-p38, ERK1/2, phospho-ERK1/2, and poly(ADP)-ribose polymerase (PARP) antibody were from New England Biolabs (Frankfurt am Main, Germany). AdipoR1 and AdipoR2 antibodies were used as recently described (24, 40). AdipoR1 (CCUCUUA-GAGAAUUCAGUAtt) and AdipoR2 (CCAACUGGAUGGUACAC GAtt) human small-interfering RNAs (siRNAs) and Silencer Negative Control 1 siRNA were from Applied Biosystems (Darmstadt, Germany). The p38 MAPK inhibitor SB-203580, the ERK1/ERK2 inhibitor PD-98059, the JAK2 inhibitor AG-490, the InSolution NF- κ B Activation Inhibitor, the STAT3 inhibitor peptide, and STAT3 Inhibitor VI (SI_VI) were from Calbiochem (Darmstadt, Germany).

Primary hepatocytes and hepatocytic cell lines. Tissue samples from liver resections were obtained from patients undergoing partial hepatectomy for metastatic liver tumors of colorectal cancer. Experimental procedures were performed according to the guidelines of the charitable state controlled foundation HTCR (Human Tissue and Cell Research), with the informed patient's consent approved by the local ethical committee of the University of Regensburg (35). Primary human hepatocytes were isolated and cultivated in serum-free medium (DMEM supplemented with 4.5 g/l glucose, 0.4 ng/ml hydrocortisone, 0.415 mU/ml insulin, 2 mM glutamine, and 100 U/ml penicillin/streptomycin) as previously described (41).

Contaminating cells using the standard isolation protocol (11) are mainly Kupffer cells and endothelial cells and are <2% as examined by light microscopy and RT-PCR studies (11). In the highly purified hepatocyte fraction, no contaminating cells were detected using light microscopy, indicating <0.1% contaminating cells, and this is in accordance with results from RT-PCR studies (11) where CD68 as a marker of Kupffer cells was not amplifiable. CD31 as a marker for endothelial cells was amplified by RT-PCR but was shown to be similarly abundant in hepatocyte fractions isolated by the standard protocol and fractions following an additional purification step with Percoll indicating that mainly Kupffer cells are removed by this purification step.

The hepatocellular carcinoma cell lines Hep G2, Hep 3B, and PLC were obtained from the American Type Culture Collection (Wesel, Germany) and were cultivated in RPMI medium (GIBCO-BRL, Karlsruhe, Germany) supplemented with 10% FCS as described (25).

Electroporation. Cells (2×10^6) were suspended in 100 μ l Nucleofector Solution (Mouse Hepatocyte Nucleofector Kit, Lonza, Wuppertal, Germany), and 3 μ g siRNA was added. Electroporation was performed using Nucleofector I (Lonza, Wuppertal, Germany) and the program T16. Immediately after transfection, 500 μ l medium supplemented with 10% FCS, 50 mM trehalose, and 1.5% dimethyl sulfoxide were added. The resuspended hepatocytes were transferred to 2.4 ml medium supplemented with 10% FCS and 50 mM trehalose and cultivated in Collagen I Cellware six-well plates (BD Biosciences,

Heidelberg, Germany) until used in the experiments. Before stimulation with adiponectin or IL-6, cells were cultivated for 12 h in serum-free medium.

SDS-PAGE and immunoblotting. SDS-PAGE and immunoblotting were performed as recently described (24, 25, 40). The hepatocytes were solubilized in RIPA buffer. Proteins (10 μ g) were separated by SDS-PAGE and were transferred (80 volts, 90 min) to polyvinylidene difluoride membranes (Bio-Rad, München, Germany). Incubations with antibodies (diluted 1:1,000-fold) were performed in 1.5% BSA in PBS, 0.1% Tween overnight. Detection of the immune complexes was carried out with the enhanced chemiluminescence Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany).

Real-time RT-PCR. Real-time RT-PCR was performed as described elsewhere (40). The specificity of the PCRs was confirmed by sequencing of the PCR fragments (Geneart, Regensburg, Germany). The primers for β -actin were 5'-CTA CGT CGC CCT GGA CTT CGA GC-3' (β -actin uni) and 5'-GAT GGA GCC GCC GAT CCA CAC GG-3' (β -actin reverse). CXCL8 was amplified with 5'-ACC GGA AGG AAC CAT CTC ACT G-3' (CXCL8 uni) and 5'-GCA TCT GGC AAC CCT ACA ACA-3' (CXCL8 reverse).

Enzyme-linked immunosorbent assay. The enzyme-linked immunosorbent assays (ELISAs) were performed as recommended by the distributor. Supernatants were diluted 1:500 for determination of CXCL8. All measurements were performed in duplicate. Total protein concentrations were determined in the hepatocytes incubated with or without adiponectin (4 different experiments) and were 1.6 ± 0.3 μ g/ μ l, indicating variations of maximally 20% that are explained by differences in media volume or cell number, and, therefore, were not taken into account for calculation of CXCL8 concentrations in the supernatants.

Electrophoretic mobility shift assay. Nuclear protein was isolated as recently described (31). The Lightshift Chemiluminescent Kit (Thermo Fisher Scientific, Bonn, Germany) was used according to the instructions of the manufacturer. An oligonucleotide duplex containing a NF- κ B binding site (AGTTGAGGGGACTTCCCAGG) or a mutated NF- κ B binding sequence (AGTTGAGGcGACTTCCCAGGC) was used.

Statistics. Data are given as mean values \pm SD (SPSS 15.0 for Windows). Statistical differences were analyzed by two-tailed *t*-test, and a value of $P < 0.05$ was taken as statistically significant.

RESULTS

Adiponectin induces CXCL8 in primary human hepatocytes. Primary human hepatocytes of eight different donors were isolated, cultivated for 48 h, and subsequently incubated with 10 μ g/ml adiponectin for 24 h. CXCL8 mRNA and β -actin mRNA for normalization were determined by real-time RT-PCR, and CXCL8 mRNA was found to be nearly eightfold induced by adiponectin (Fig. 1A). The protein was measured in the supernatants of these cells, and CXCL8 was found to be significantly higher in the supernatants of adiponectin-treated cells (Fig. 1B). Incubation of hepatocytes of three different donors with adiponectin for 3, 6, and 12 h indicated that CXCL8 protein was already induced after 6 h and was not further upregulated in cells stimulated for 12 h (Fig. 1C). When primary hepatocytes of three different donors were cultivated in the presence of increasing concentrations of adiponectin for 24 h, a significant upregulation of CXCL8 was observed by 10 μ g/ml adiponectin, whereas 0.1, 1, 2.5, and 5 μ g/ml had no effect (Fig. 1D).

To analyze the contribution of nonparenchymal cells to the effects of adiponectin, CXCL8 was determined in the supernatants of hepatocytes isolated with the standard protocol (98% hepatocytes in cell fraction) and in more highly purified hepa-

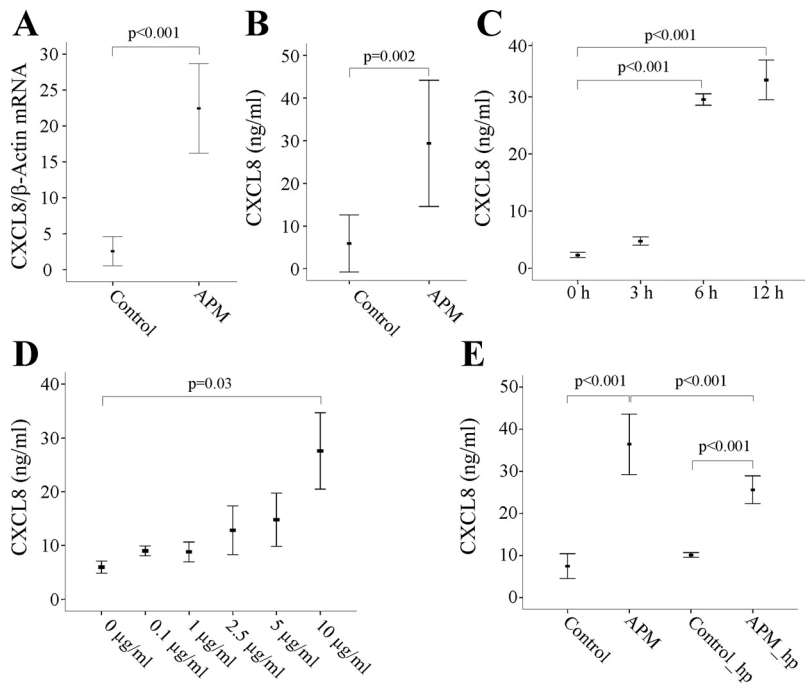


Fig. 1. Adiponectin induces CXCL8 in primary human hepatocytes. **A:** CXCL8 mRNA and β -actin mRNA used for normalization were determined in primary human hepatocytes incubated with adiponectin. **B:** CXCL8 was determined in the supernatant of primary hepatocytes incubated with 10 μ g/ml adiponectin (APM) for 24 h. **C:** CXCL8 was determined in the supernatant of primary hepatocytes incubated with 10 μ g/ml adiponectin for 0, 3, 6, and 12 h. **D:** CXCL8 was determined in the supernatant of hepatocytes incubated with increasing amounts of adiponectin for 24 h. **E:** CXCL8 was determined in the supernatants of hepatocytes purified with the standard procedure (\sim 2% contaminating cells) and highly pure hepatocytes (hp, less than 0.1% contaminating cells) of the identical donors and cultivated with or without adiponectin for 24 h.

toocytes (<0.1% contaminating cells) from the identical donor (11). CXCL8 concentration was similar in the supernatants of the hepatocytes isolated by the standard procedure and the supernatants of cells undergoing an additional Percoll purification. However, adiponectin-stimulated CXCL8 was lower in the supernatants of highly purified hepatocytes, indicating that adiponectin also upregulated CXCL8 in the nonparenchymal cells (Fig. 1E).

To exclude any toxic effects of the recombinant adiponectin, lactate dehydrogenase was measured in the supernatants but was found to be similar in control and adiponectin-treated hepatocytes (data not shown). Furthermore, the nuclear PARP that is cleaved by caspases in response to environmental stress was similarly abundant in control and adiponectin-treated cells (data not shown).

AdipoR1 is involved in adiponectin-mediated upregulation of CXCL8. Primary human hepatocytes (triplicate experiments with hepatocytes of three different donors) were transfected with scrambled siRNAs as control or siRNAs specific for AdipoR1 or AdipoR2 by electroporation. After transfection (48

h), the specific siRNAs had reduced AdipoR1 expression by \sim 90% and AdipoR2 by \sim 80% when compared with the control siRNA-treated cells (Fig. 2A). CXCL8 levels were similar in the supernatants of control siRNA-, AdipoR1 siRNA-, and AdipoR2 siRNA-treated hepatocytes. CXCL8 in the supernatants of hepatocytes with AdipoR2 knockdown and adiponectin stimulation was similar to controls but was significantly lower in the supernatant of AdipoR1 siRNA-treated cells incubated with adiponectin (Fig. 2B).

Adiponectin does not induce CXCL8 in hepatocytic cell lines. CXCL8 was determined in the supernatants of Hep G2, Hep 3B, and PLC cell lines cultivated for 24 h. The levels were similar in Hep G2 cells, Hep 3B cells, and primary hepatocytes and were significantly higher in the supernatants of PLC cells (data not shown). However, adiponectin did not induce CXCL8 in the cell lines even when 20 μ g/ml recombinant protein was used (data not shown).

Adiponectin enhances NF- κ B activity in primary hepatocytes. Induction of CXCL8 may depend on the activation of NF- κ B; therefore, NF- κ B activity was analyzed by electro-

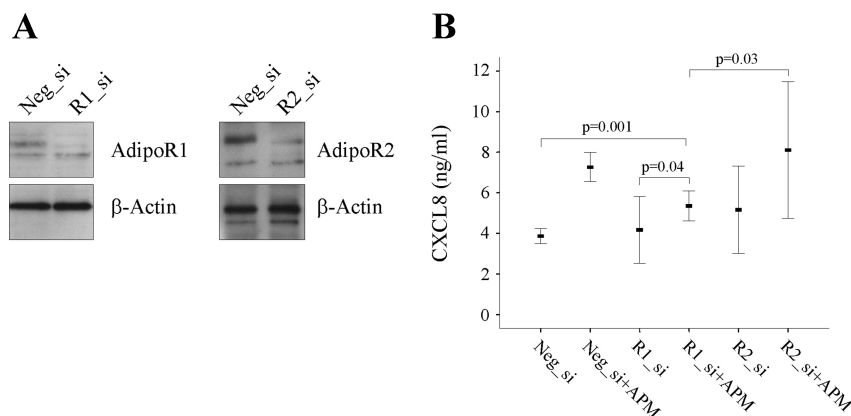


Fig. 2. Adiponectin induces CXCL8 via adiponectin receptor (AdipoR) 1. **A:** immunoblot with cell lysates of primary hepatocytes treated with small-interfering RNA (siRNA) to knock down AdipoR1 or AdipoR2 or control siRNA (Neg_si). **B:** CXCL8 in the supernatants of primary hepatocytes pretreated with siRNAs to knock down AdipoR1 or AdipoR2 (Neg_siRNA as control) and incubated with adiponectin.

phoretic mobility shift assay using nuclear protein of primary hepatocytes or Hep G2 cells that were incubated with adiponectin for 0, 30, or 60 min. An enhanced binding to an oligonucleotide duplex containing an NF- κ B binding site was only observed when nuclear protein of primary cells stimulated with adiponectin was investigated. Binding was not detected when an oligonucleotide duplex with a mutated NF- κ B site was used, indicating that the observed effect was specific (Fig. 3A). Binding to NF- κ B was not enhanced in Hep G2 cells incubated with adiponectin for 30 or 60 min (data not shown). In accordance with elevated NF- κ B activity, in nuclear extracts of adiponectin-incubated primary hepatocytes, the abundance of the NF- κ B family member p65 was transiently elevated (Fig. 3B). The InSolution NF- κ B Activation Inhibitor was used to investigate the involvement of NF- κ B, and 5 μ M of the inhibitor nearly blocked CXCL8 induction by adiponectin, whereas basal concentrations were not altered (Fig. 3C).

The p38 MAPK and ERK1/2 are involved in CXCL8 induction by adiponectin. Adiponectin stimulated the phosphorylation of the p38 MAPK within 30 min of incubation (Fig. 3D). Because p38 MAPK may be involved in the induction of CXCL8, hepatocytes were incubated with 10 μ g/ml adiponectin alone or in combination with the p38 MAPK inhibitor SB-203580 at a concentration of 100 or 200 nM. CXCL8 in the supernatants of hepatocytes incubated with the p38 MAPK inhibitor alone was significantly reduced (Fig. 3E and data not shown). Adiponectin-mediated elevation of CXCL8 was slightly lower when 200 nM of the inhibitor was added (Fig. 3E).

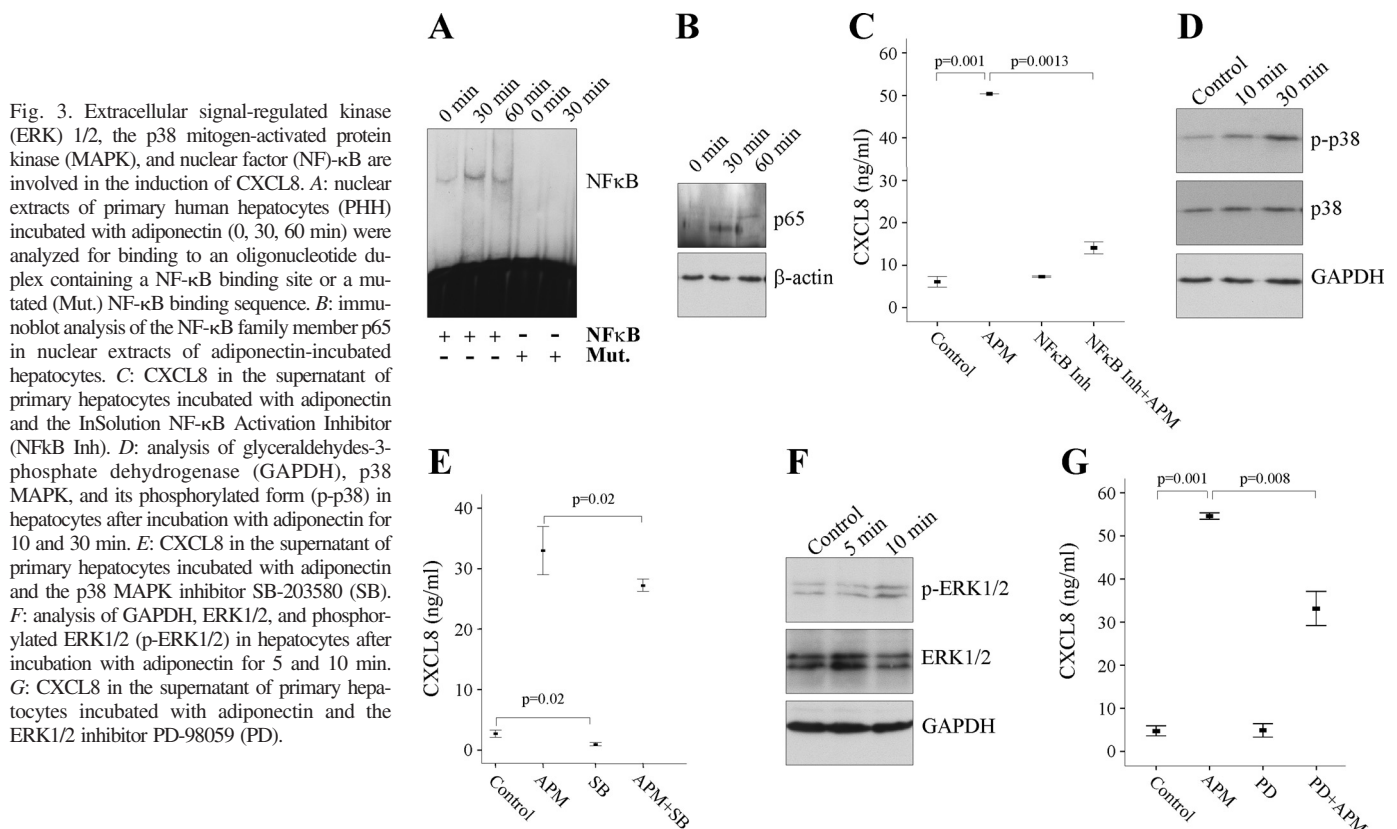
Adiponectin stimulated the phosphorylation of ERK1/2 10 min after addition of 10 μ g/ml adiponectin to the medium (Fig.

3F). PD-98059 (20 μ M), an inhibitor of the ERK1/2 pathway, significantly reduced CXCL8 in the supernatant of adiponectin-stimulated cells but did not alter CXCL8 release in control-cultivated hepatocytes (Fig. 3G).

Adiponectin stimulates the phosphorylation of STAT3. Activation of STAT3 was described to induce CXCL8; therefore, it was analyzed whether adiponectin enhances STAT3 phosphorylation. Elevated phosphorylated STAT3 was detected in primary hepatocytes after 3 and 6 h incubation with adiponectin (Fig. 4A). IL-6 activates STAT3; therefore, IL-6 was determined in supernatants of hepatocytes of eight donors incubated with or without adiponectin for 24 h. IL-6 was 42.0 ± 25.4 pg/ml ($P = 0.003$) in the supernatants of adiponectin-incubated cells and nearly eightfold induced compared with control-cultivated hepatocytes (Fig. 4B). IL-6 (100 pg/ml) did not stimulate phosphorylation of STAT3 in primary hepatocytes, indicating that these low IL-6 levels may not account for the activation of STAT3 in the cells (Fig. 4C). However, this concentration of IL-6 may act synergistically with adiponectin in stimulating activation of P-STAT3, but P-STAT3 was similar in hepatocytes incubated with adiponectin alone or in combination with 100 pg/ml IL-6 (Fig. 4D).

In Hep G2 cells, adiponectin (10 μ g/ml) could not enhance STAT3 phosphorylation, although IL-6, a well-described activator of STAT3, markedly elevated P-STAT3 (data not shown).

To analyze whether adiponectin-mediated activation of STAT3 is involved in the induction of CXCL8, AG-490, a JAK family tyrosine kinase inhibitor described to inhibit activation of STAT3 (17), was applied. However, AG-490 used in the nM and μ M range did not influence CXCL8 in adiponectin-incu-



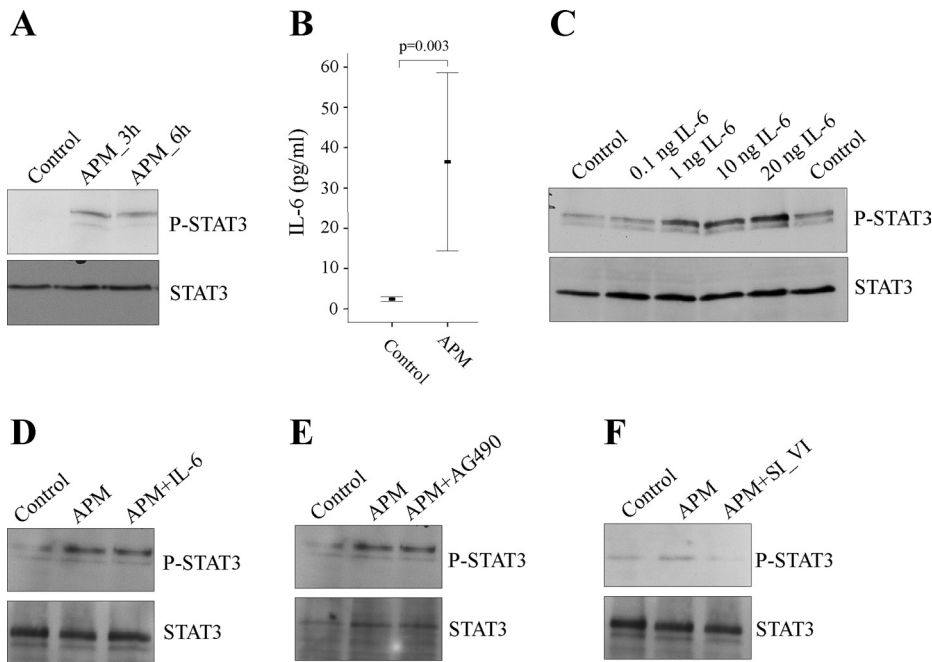


Fig. 4. Adiponectin activates STAT3. *A*: P-STAT3 and STAT3 in cell lysates of primary human hepatocytes treated with adiponectin. *B*: interleukin (IL)-6 in the supernatant of hepatocytes cultivated with or without adiponectin for 24 h. *C*: P-STAT3 and STAT3 in primary human hepatocytes treated with different concentrations of IL-6. *D*: P-STAT3 and STAT3 in primary human hepatocytes treated with adiponectin alone or in combination with 0.1 ng/ml IL-6. *E*: P-STAT3 and STAT3 in cell lysates of primary human hepatocytes treated with adiponectin alone or in combination with AG-490. *F*: P-STAT3 and STAT3 in cell lysates of primary human hepatocytes treated with adiponectin alone or in combination with STAT3 Inhibitor VI (SI_VI).

bated cells (data not shown), and P-STAT3 was similar in hepatocytes incubated with adiponectin or adiponectin and 1 μ M AG-490 (Fig. 4E). Furthermore, the inhibitor was analyzed in IL-6-incubated hepatocytes and failed to abolish IL-6-stimulated phosphorylation of STAT3 (data not shown). Therefore, SI_VI (0.4 mM), which directly prevents STAT3 phosphorylation, was used. SI_VI (0.4 mM) reduced P-STAT3 in IL-6-treated hepatocytes (data not shown) and in adiponectin-stimulated cells (Fig. 4F). Adiponectin and SI_VI separately enhanced CXCL8 in the supernatants, and coincubation of the hepatocytes with SI_VI and adiponectin led to a synergistic elevation of CXCL8 (Fig. 5A). Similar results were obtained when STAT3 was blocked by a STAT3 Inhibitor Peptide (0.2 mM) (Fig. 5B). These findings indicate that activated STAT3 inhibits CXCL8 release in hepatocytes. Therefore, the influence of IL-6 on hepatocytic CXCL8 was determined, and it was shown that 20 ng/ml IL-6 slightly but significantly reduced CXCL8 in the supernatants (Fig. 5C).

DISCUSSION

In the current study, we demonstrated that adiponectin induces CXCL8 mRNA and protein in primary human hepa-

toocytes. As expected, basal CXCL8 release in the primary cells showed some variation but, although consistently observed, the effect of adiponectin ranged between a 2- to 15-fold induction of CXCL8, indicating that intrinsic properties of the primary cells alter the response to adiponectin in vitro.

Elevated CXCL8 stimulated by adiponectin was already demonstrated in human microvascular endothelial cells, rheumatoid synovial fibroblasts, and monocytes (13, 29, 40). The p38 MAPK, JNK, and NF- κ B were identified to participate in the adiponectin-mediated increase of CXCL8 in these cells. The CXCL8 promoter contains a NF- κ B binding site (9), and inhibition of NF- κ B nearly blocked induction of CXCL8 in the current study. Furthermore, an enhanced binding to a NF- κ B binding site was detected in adiponectin-treated primary hepatocytes and is in accordance with an increased nuclear translocation of the p50 and p65 NF- κ B family members demonstrated by others (13), and for p65 herein. Activation of NF- κ B by adiponectin has already been shown in endothelial cells, fibroblasts, and myocytes (34, 36, 38) but to the best of our knowledge not in hepatocytes so far. NF- κ B is a master regulator of inflammation, cell survival, and proliferation. In NEMO^{LPC-KO} mice, NF- κ B activity in the liver is completely

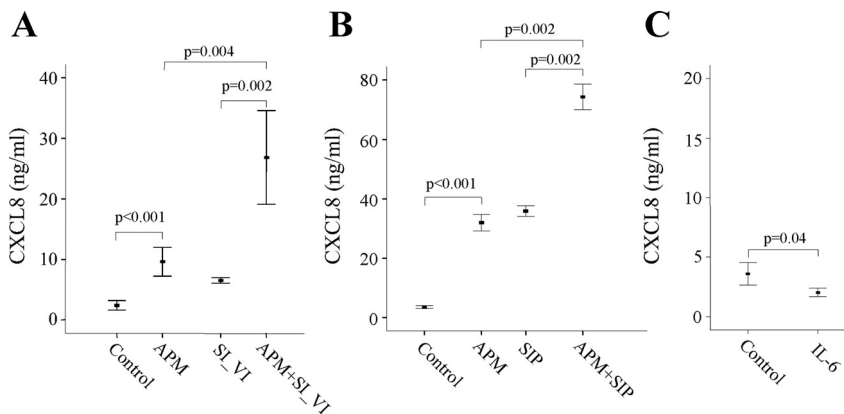


Fig. 5. Inhibition of STAT3 elevates CXCL8. *A*: CXCL8 in the supernatant of hepatocytes incubated with adiponectin alone, SI_VI alone, or both for 24 h. *B*: CXCL8 in the supernatant of hepatocytes incubated with adiponectin alone, STAT3 Inhibitor Peptide (SIP) alone, or both for 24 h. *C*: CXCL8 in the supernatant of hepatocytes incubated with IL-6.

blocked, and the specific loss of NEMO in hepatocytes spontaneously induces chronic inflammation, steatohepatitis, and liver cancer (18). Therefore, adiponectin-mediated activation of NF- κ B in liver parenchymal cells may resemble one mechanism that explains the hepatoprotective effects of this adipokine (10, 32). The p38 MAPK that participates in the adiponectin-stimulated induction of CXCL8 in monocytes and of IL-6 in fibroblast (34, 40) is also partly involved in hepatocytic upregulation of CXCL8. This kinase is activated by binding of adiponectin to AdipoR1 (34), and, in accordance with these data, knock down of AdipoR1 reduced CXCL8 protein in the supernatants of adiponectin-treated primary hepatocytes. However, in synovial fibroblasts, AdipoR2 was involved in CXCL8 induction, whereas in human microvascular endothelial cells the effect was independent of both adiponectin receptors described so far (13, 29). These studies confirmed RNAi-mediated knock down of AdipoR1 or AdipoR2 at the mRNA but not the protein level; therefore, the extent of protein reduction in their and the current studies cannot be compared but might partly explain the discrepant findings (13, 29). Pharmacological inhibition of ERK1/2 also reduced CXCL8 in the supernatants of adiponectin-treated hepatocytes, indicating that p38, ERK1/2, and NF- κ B are involved in the induction of CXCL8 by adiponectin.

Upregulation of CXCL8 by adiponectin indicates proinflammatory effects of this adipokine although several studies demonstrate that adiponectin is an anti-inflammatory protein. Nevertheless, elevated circulating adiponectin levels that even correlate with inflammatory markers were found in patients with inflammatory bowel disease or type 1 diabetes, and an induction of inflammatory proteins similar to the findings herein was described in several studies (4). Therefore, the link between adiponectin and inflammation is unclear, and additional studies have to clarify the role of adiponectin in inflammatory diseases.

Most studies published so far described that activation of STAT3 is associated with an elevation of CXCL8. Nonetheless, activated STAT3 was also identified as a repressor of inflammatory responses and CXCL8 synthesis (5, 20). STAT3 inhibits NF- κ B activity by directly binding to this transcription factor, and this may explain the elevated synthesis of cytokines and chemokines in STAT3-deficient cells (42, 47). Adiponectin activates STAT3 in primary hepatocytes, and a similar effect was very recently demonstrated in mouse fibroblasts (17). Inhibition of STAT3 significantly elevated CXCL8 release in control and adiponectin-incubated hepatocytes, indicating that activated STAT3 inhibits CXCL8 synthesis in the cells studied herein. Whether this repressive effect is explained by STAT3-mediated downregulation of NF- κ B activity (47) has to be evaluated in future studies. In accordance with a repressive effect of activated STAT3 on CXCL8, IL-6 also lowered hepatocytic CXCL8. Inhibition of JAK2 neither influenced adiponectin-mediated induction of CXCL8 nor IL-6-stimulated activation of STAT3, and this finding is in accordance with studies in JAK2-deficient mice where IL-6 signaling was not affected (27).

In contrast to primary hepatocytes, hepatocytic cell lines were resistant to the effects of adiponectin, and neither an elevated release of CXCL8 nor an activation of STAT3 was observed. To exclude the possibility that solely nonparenchymal cells respond to adiponectin, highly purified hepatocytes

with <0.1% contaminating cells were used (11). Although the induction of CXCL8 was less pronounced in these cell preparations when compared with less pure hepatocyte fractions with ~2% nonparenchymal cells isolated from the identical liver, adiponectin clearly enhanced CXCL8 levels.

Although Hep G2 cells did not respond to adiponectin incubation, P-STAT3 was elevated in Hep G2 cells incubated with IL-6, indicating that these cells have a proper response to a well-described activator of STAT3. Hep G2 cells have been described to respond to recombinant adiponectin, but significant effects were only observed when 30 μ g/ml recombinant protein were used (21, 22). These high amounts of protein even repressed STAT3 activity in Hep G2 cells, but CXCL8 was not determined in this study (22).

Activation of STAT3 in adult mouse cardiac fibroblasts by adiponectin was explained by an autocrine/paracrine mechanism where adiponectin upregulated IL-6 to a concentration of ~1 ng/ml in the supernatants of the cells, and IL-6 subsequently activated STAT3 (11). However, IL-6 levels in the supernatants of the purified primary hepatocytes were very low (<50 pg/ml) and most likely originate from nonparenchymal cells (7), indicating that different mechanisms are involved in the activation of STAT3 in hepatocytes and fibroblasts.

Adiponectin decreases CXCL8 release in tumor necrosis factor-activated human aortic endothelial cells (15) and in lipopolysaccharide-stimulated macrophages, whereas CXCL8 is induced in nonactivated cells (30). These findings might indicate that, in inflammation, NF- κ B stimulating pathways of adiponectin are suppressed and anti-inflammatory signals are transduced. Therefore, adiponectin-mediated activation of STAT3 may prevail in these conditions and contribute to the suppression of CXCL8. These findings may explain the dual role of adiponectin described as a pro- and anti-inflammatory protein in various studies (4, 29, 30, 40).

To summarize, in the current study, it was demonstrated that adiponectin induces CXCL8 by a pathway involving the p38

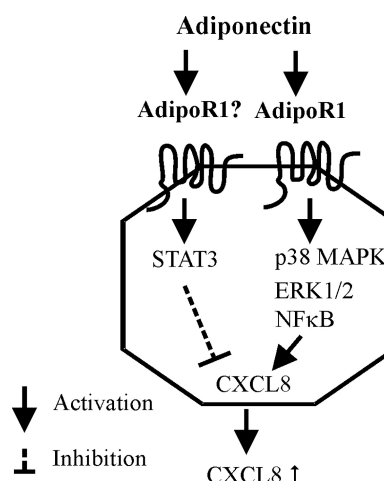


Fig. 6. Adiponectin activates the p38 MAPK, ERK1/2, and NF- κ B and thereby elevates CXCL8 in the supernatants of primary human hepatocytes. In addition, adiponectin stimulates phosphorylation of STAT3, and activated STAT3 inhibits induction of CXCL8. Nevertheless, the net result is 2- to 8-fold elevated CXCL8 in adiponectin-incubated hepatocytes. Involvement of AdipoR1 in the induction of CXCL8 was confirmed by siRNA experiments. Activation of STAT3 may also depend on AdipoR1 because siRNA-mediated knock down of AdipoR2 did not alter CXCL8 but should be associated with elevated CXCL8 if STAT3 is activated by this receptor.

MAPK, ERK1/2, and NF- κ B. In addition, adiponectin activates STAT3 and thereby suppresses CXCL8 release (Fig. 6). CXCL8 is an antiapoptotic protein, and, therefore has to be tightly regulated to guarantee cell survival but also to prevent excess cell proliferation that may lead to carcinogenesis.

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